For Reference

NOT TO BE TAKEN FROM THIS ROOM

For Reference

NOT TO BE TAKEN FROM THIS ROOM

Ex libris universitatis albertaensis

UNIVERSITY OF ALBERTA

QUAECUMQUE VERA

University of Alberta
Printing Department

Digitized by the Internet Archive
in 2019 with funding from
University of Alberta Libraries

https://archive.org/details/Wu1965_0

THE UNIVERSITY OF ALBERTA

RELATIONSHIPS BETWEEN METABOLIZABLE ENERGY AND PROTEIN LEVELS OF RATIONS AND THE LYSINE REQUIREMENT OF CHICKS

by

SHIRLEY I-SHIEN WU

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

DEPARTMENT OF ANIMAL SCIENCE

EDMONTON, ALBERTA

NOVEMBER, 1965

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Relationships Between Metabolizable Energy and Protein Levels of Rations and the Lysine Requirement of Chicks" submitted by Shirley I-Shien Wu, B.S., in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

An investigation was undertaken to determine relationships that may exist between metabolizable energy and protein levels of rations and the lysine requirements of starting chicks. Four feeding trials, each of four weeks duration, were conducted using day-old Dominant White x White Plymouth Rock chicks. The trials were factorially designed; each trial involved rations with three levels of metabolizable energy (1080, 1260, and 1440 Calories per pound) and four levels of lysine (0.9, 1.0, 1.1, and 1.2 per cent) at each energy level. In Trials I, II, and III, rations containing 18, 21, and 24 per cent of protein, respectively, were used. In Trial IV, the rations were similar to those used in Trials I and III except that supplementary arginine and methionine were added to all rations. Metabolizable energy, protein, and lysine levels were determined on the experimental rations. Records were kept on body weight and feed consumption.

The results of the first three trials indicated, in general, that increasing the metabolizable energy content from 1080 to 1260 to 1440 Calories per pound in chick starters containing 18, 21, or 24 per cent of protein and 0.9 per cent of lysine had no effect on the growth rate of chicks to four weeks of age. When the rations containing 18 and 24 per cent of protein were supplemented with arginine and methionine, increasing the energy level of the rations resulted in increased rate of growth. It therefore appears that the basal starters employed in the first three trials may have been limiting in arginine and methionine, thus preventing changes in growth rate as the energy level was increased.

The effect of the addition of lysine to the ration was variable.

An increase in rate of growth from lysine supplementation was noted in Trial III with rations containing 1260 and 1440 Calories of metabolizable energy per pound and, in Trial IV, with the rations containing the highest levels of energy in both the low and high-protein series. In most instances where a response occurred, 1 per cent lysine in the ration was found as effective for growth promotion as higher levels. This suggests that the optimum Calories of metabolizable energy:lysine ratio is approximately 1440:1 rather than 1200:1 as was assumed.

The data obtained indicated that the level of energy in the rations was the main factor affecting feed efficiency. In general, as energy levels of the rations were increased the amount of feed required per unit of gain was decreased. The addition of lysine to the rations, at any one protein or energy level, had no significant effect on efficiency of feed utilization.

ACKNOWLEDGMENTS

The author wishes to thank Dr. L. W. McElroy, Head of the Department of Animal Science, for placing the facilities of the department at her disposal. The encouragement, direction, and criticism given by Dr. A. R. Robblee, Professor of Poultry Nutrition, throughout the course of this study, and the guidance given by Dr. D. R. Clandinin, Professor of Poultry Nutrition, in the preparation of the manuscript are deeply appreciated.

The writer is also indebted to Dr. Ruth Renner, Associate Professor of Household Economics, for direction in connection with the analytical procedures, and to Dr. R. T. Hardin, Assistant Professor of Poultry Genetics, for assistance with statistical analysis of the data.

A research assistantship provided by the National Research Council is gratefully acknowledged.

TABLE OF CONTENTS

Page

LIST OF TABLES

INTRODUCTION

Many changes in poultry production have been made in recent years. This has resulted in the evolution of a poultry industry designed to provide the consumer with meat and eggs produced with the greatest efficiency possible. Although numerous factors have contributed to the progress that has been achieved, advances in nutrition should, perhaps, be credited for a major contribution to improved efficiency of production.

Changes in concepts relating to energy nutrition have been responsible for some notable advances in efficiency of poultry production. Recognition that feed ingredients varied in productive and metabolizable energy content led to development of high-energy rations. Increasing the energy level of rations resulted in decreased feed intake and increased efficiency of feed conversion which in turn gave rise to the concept that chickens adjust feed intake so that just enough feed is consumed to meet their energy requirements. This in turn led to the hypothesis that, to achieve optimum efficiency, levels of protein, minerals, and vitamins in rations for poultry should be related to the productive or metabolizable energy content of the ration. Because requirements for proteins are, in fact, requirements for amino acids, it was next thought that amino acid requirements should also be related to the energy content of the ration if optimum efficiency were to be achieved. The validity of this concept has not been thoroughly tested but there are indications that its application to poultry feeding may result in still further advances in efficiency of production.

The possibility that amino acid requirements may be directly related to the energy content of rations raises a question as to the importance of specifying exact protein requirements, provided that the requirements for individual amino acids are met. Since, of the essential amino acids, lysine is often limiting in practical poultry rations, a series of experiments was undertaken to study some of the interrelationships that may exist among metabolizable energy, protein, and lysine levels in rations for starting chicks.

REVIEW OF LITERATURE

History

Recognition of the role of energy level of the feed in improving efficiency of poultry production has led to many studies on energy nutrition. The results obtained in the studies have been responsible for changes in many nutritional concepts.

Understanding of the role of energy in poultry nutrition has developed, more or less, in three stages. The first stage was concerned with the level of energy in poultry rations. In this stage, rations of relatively high energy content were prepared by substituting high-energy grains such as corn and wheat for coarse grains and grain by-products. It was noted that increased rate of growth and improved feed efficiency resulted. The second stage involved further increases in the energy content of rations by the use of surplus fats which became available at prices which permitted their inclusion in poultry rations. The higher concentrations of energy in feeds attained by the use of fat resulted in improved productive performance in some cases but not in others. The third stage has included studies designed to determine some of the interrelationships that exist between energy concentrations and levels of nutrients in rations.

Interest in energy utilization by poultry was stimulated by a series of extensive studies conducted by investigators at Texas Agricultural and Mechanical College (Fraps and Carlyle, 1939; Fraps et al., 1940; Fraps and Carlyle, 1942; Fraps, 1945; Fraps, 1946). They noted that feedstuffs varied widely in energy content, the more fibrous feeds being generally lower in energy. Scott et al. (1947) replaced low-energy grains and grain by-products with high-energy ingredients in

a broiler ration and observed that the higher energy ration gave faster growth and more efficient feed utilization. Similar results were obtained by Panda and Combs (1950), Leong et al., (1955), and Donaldson et al., (1956).

It soon became apparent that on an ad libitum feeding system chickens normally tend to eat enough feed to meet their requirements for energy. Hill and Dansky (1954) observed that increasing energy concentrations in the diet were accompanied by decreased feed intake, improved feed efficiency, and more rapid growth rate. Peterson et al. (1954) also noted that when the energy content of a ration decreased, feed intake increased to satisfy the energy needs of the chicks. It was, however, observed that when the fibre content of the ration was very high, chickens were unable to get enough feed to meet their energy requirements. The inability of chickens to consume sufficient amounts of fibrous feeds to meet their energy needs has resulted in a recommendation that rations for broilers and for starting and growing chickens should contain a minimum of 1430, 1215, and 1095 Calories of metabolizable energy per pound of feed, respectively (Combs, 1962).

Energy-Protein Relationships

The increasing use of energy values of feed ingredients in formulating poultry rations has focused attention on studies of relationships between energy concentration and levels of other nutrients in the ration. This has come about because, on many occasions, increasing the energy content of rations did not give the improvements in growth rate and feed efficiency that were expected. Hill and Dansky (1950) observed that when a high-energy, low-protein ration was fed, growth rate was depressed, but when the energy level of the ration was also lowered,

normal growth was restored. Biely and March (1954) noted that only when the protein content of the ration was sufficient would increased levels of energy in the ration give improved feed efficiency.

Much research has been conducted since 1954 on relationships between energy content of the ration and protein requirements of poultry. The studies showed, in general, that as the energy content of the ration was increased the percentage of protein in the ration should also be increased if optimum feed efficiency and growth rate were to be obtained. Combs and Romoser (1955) suggested that a ratio should be maintained between the productive energy concentration and the crude protein level of the ration. Their experiments indicated that this ratio influenced feed intake, growth rate, and feed conversion of growing birds. They introduced the term Calorie:protein (C/P) ratio which was defined as the Calories of energy per pound of ration divided by the percentage of protein in the ration. They suggested that the optimum C/P ratio for broilers was 42 Calories of productive energy. Subsequent work has indicated that a C/P ratio lower than the optimum had less effect on growth rate of chicks than a wider ratio (Donaldson et al., 1956; Richardson et al., 1956; Sunde, 1956). Sibbald et al. (1961b) reported that the C/P ratio of the diet, based on metabolizable energy values, exerts considerable effect on the weight gain, feed efficiency, and metabolizable energy consumed per gram of weight gain by growing chicks. It was noted that weight gain was proportionally increased as the protein consumption increased, but an increased metabolizable energy consumption caused an opposite result. The algebraical equation derived from their data indicated that the optimal C/P ratio for maximum weight gain was 57.6; this was also the optimal ratio for

the most efficient utilization of the feed.

Many reports appearing in the literature on energy-protein balance gave results that varied depending upon the basal rations that were used and the age of the chickens. Combs and Romoser (1955) observed that C/P ratios of about 42 Calories of productive energy were optimal for broilers to five weeks of age. Guttridge (1957) reported that ratios of 44:1 and 56:1 gave maximum growth in chicks to six and eight weeks of age, respectively. Hill and Renner (1957) noted that the optimum C/P ratio, using metabolizable energy values, for starting chickens was 60 to 64. Vondell and Ringrose (1958) and Beilharz and McDonald (1959) also reported that the optimum C/P ratio was influenced by the rations used. Combs (1962) reviewed the earlier studies and tabulated the optimum C/P ratio for chickens at various ages. The results indicated a range of values that was optimum for each age and higher C/P ratios for older birds. It was suggested that the wide range of values may be attributed to variation in the nutrient content of the feedstuffs used by the different investigators. The wider ratios for older birds reflects a decrease in the requirements for protein as the birds age.

Levels of protein and energy in the ration interact with each other and to some extent influence the nutritive value of the ration. Sibbald et al. (1961a) noted that the level of protein and fat included in the ration exerted significant effects upon metabolizable energy values. An interaction among protein level, fat level, and utilization of the energy component of the fats was noted.

Baldini (1961) showed that the metabolizable energy content of a ration may be influenced by a dietary deficiency; deficient diets

proved to have more metabolizable calories per pound than adequate diets. Their data suggested that the deficient birds were able to metabolize calories efficiently but were unable to store them. Birds consuming adequate diets stored more calories from the rations; thus, improved growth rate and feed efficiency were obtained.

Energy level of the ration seems to have a greater effect on the utilization of feed by the chicken than does the protein content. O'Neil et al. (1962) observed that an excess of productive energy in relation to the amount of protein in the diet depressed rate of growth and decreased efficiency of feed utilization. An excess of protein in relation to productive energy, while not adversely affecting either growth or feed efficiency, did result in wastage of the protein portion of the ration.

Energy, Protein, and Amino Acid Interrelationships

Since the protein quality of rations may vary considerably, it soon became apparent that the use of C/P ratios was not a very precise way of expressing optimum relationships. Because protein requirements are really requirements for individual amino acids, it seemed logical that the relationships between energy level of the ration and amino acid content should be considered. Thus, the concept of Calorie:amino acid ratios was introduced. Baldini and Rosenberg (1955) showed that when the chick's methionine requirement was expressed as a percentage of the ration, it increased directly as the energy content of the ration increased. Schwartz et al. (1958) demonstrated that a linear relationship existed between the energy content of the ration and the chick's requirement for lysine. The experiments confirmed and extended the

previous observation of Williams and Grau (1953) who noted that when sufficient energy was supplied in the ration, increased lysine intake resulted in more rapid growth.

Although experiments to support the concept that amino acid requirements are primarily controlled by the energy content of the ration have been reported, there is evidence that amino acid requirements may also be affected by the level of protein in the ration. Evidence has been presented (Grau and Kamei, 1950; Almquist, 1952; Griminger et al., 1956) which indicates that amino acid requirements expressed as percentages of the diet increase as the protein content of the ration increases. However, when expressed as percentages of dietary protein, amino acid requirements appear to decrease with increasing protein content of the diet.

The above reports, while indicating a possible influence of protein level of the ration on amino acid requirements, fail to delineate any effects of energy levels or energy-protein relationships. On the other hand, the importance of energy level of the diet on this relationship has been demonstrated by Rosenberg and Baldini (1956). They found that at high-energy levels the methionine requirement, expressed as a percentage of the diet, increased as protein level increased; however, at lower energy levels the methionine requirement did not increase proportionately with increased protein levels. Gordon et al. (1958) found that the amino acid requirement, as a per cent of protein, was constant over a wide range of protein content provided that the proportion of Calories to protein was kept relatively constant. This was true even though the birds grew at different rates because of differences in total nutrients. It therefore appears that the energy

level of the ration is more critical than protein level in determining optimum amino acid levels in rations for chicks.

Effects of Energy Level of the Ration on Lysine Requirement

Until very recently it has been the usual practice to express amino acid requirements of chicks as a percentage of the ration. Almquist and Mecchi (1942) reported that a level of 0.9% of lysine in chick rations was required for optimum growth. A later report (Almquist, 1947) confirmed the earlier study. A number of other reports (Grau, 1948; Grau and Kamei, 1950; Milligan et al., 1951; Richardson and Blaylock, 1950; Blaylock and Richardson, 1950) reported lysine requirements ranging from 0.86 to 1.1% of the diet. Edwards et al. (1956) noted that a level of 1.1% was required for optimum growth of fast-growing chicks with a ration containing 20.5% of protein but with slow-growing chicks the requirement appeared to be 0.9%. Griminger and Scott (1959) demonstrated that rate of growth had no effect on lysine requirement when growth, gain, or instantaneous rate of growth per day were used as criteria of lysine sufficiency. It was noted, however, that there were marked variations between experiments in optimum lysine levels ranging from 0.93 to 1.13%. After evaluating all of the evidence available, the Sub-committee on Poultry Nutrition of the National Research Council, National Academy of Sciences (1960) recommended that a level of 1% of lysine be provided to meet the requirements of chicks fed rations containing 20% of protein and 900 Calories of productive energy per pound of feed.

The hypothesis that nutrient requirements may be influenced by the energy content of the ration has led to some reassessment of amino acid requirements. Schwartz et al. (1958) observed that the chick's requirement for lysine was directly related to the energy level of the

ration. It was noted that the lysine requirements increased by 0.07% for each increase of 100 Calories of productive energy per pound of ration. Based on studies with broiler rations that performed satisfactorily, Combs (1962) suggested that the minimum lysine:energy ratio for broilers to four and one-half weeks of age was 0.78% of lysine per therm of metabolizable energy per pound of ration. In a later report, Combs and Nicholson (1963) related the lysine requirements of chicks to the metabolizable energy content of the ration and found that the amounts of lysine required for optimal growth and caloric efficiency were 3.66 and 3.93 g per therm of metabolizable energy, respectively. Thus, it would appear that expressing lysine requirements as a function of energy level of the ration may be more precise than expressing it as a percentage of the ration.

EXPERIMENTS AT THE UNIVERSITY OF ALBERTA

Experiments were designed to permit study of the effects of varying the levels of metabolizable energy and protein in the ration on rate of growth, feed conversion, and the lysine requirement of chicks.

Status of the Problem

Recent evidence (see Review of Literature) seems to indicate that amino acid requirements may be directly related to the energy level of the ration. Indeed, this relationship appears to be of even greater significance in relation to growth rate and efficiency of feed conversion than level of protein in the diet.

Considering all factors which may influence the quality of rations, it was hypothesized that (a) rations might be improved by supplementing them with the most limiting amino acids, and (b) growth rate and feed efficiency might be improved by feeding a ration balanced with respect to energy and amino acid levels. Since lysine is an amino acid often limiting in practical-type chick rations, it was deemed advisable to study the interrelationships of metabolizable energy, protein, and lysine in practical-type chick starters.

Experimental (General)

Feeding trials

Day-old male Dominant White x White Plymouth Rock chicks, hatched at the University of Alberta Poultry Research Farm, were used to conduct the feeding experiments. The chicks were individually weighed, wing-banded, and allotted 15 to a group. They were brooded in electrically heated batteries with raised screen floors. Experimental rations and

water were fed *ad libitum*. Body weight and feed consumption were recorded at weekly intervals until the chicks were four weeks old.

Four trials were conducted involving variations in protein, energy, and lysine levels of the rations. In order to do this, rations were formulated in such a way that, with each protein level used, variations in energy level and lysine level were made with a minimum effect upon other components of the diet.

In Trials I, II, and III, protein levels of 18, 21, and 24%, respectively, were employed. In order to avoid appreciably affecting the level of lysine in the basals used in Trials II and III, the additional protein required over that contained in the basal used in Trial I was supplied by zein, which contains a very low level of lysine (0.62%, Block and Weiss, 1956). Thus, the changes in protein levels were accomplished with a negligible effect upon the lysine content of the basals. In Trial IV basals similar to those used in Trials I and III, except that they were supplemented with methionine and arginine, were employed.

In each trial, rations containing three levels of energy (1080, 1260, and 1440 kcal of metabolizable energy per pound) were formulated. Adjustments in energy level were made by varying the amounts of Solka Floc, fat, and cornstarch in the ration. Substitutions with these ingredients permitted changes in energy content of the rations without affecting levels of protein, amino acids, minerals, or vitamins.

In the actual preparation of the experimental rations, a mix of ingredients common to all rations (Table 1) was used. It constituted 75% of each of the experimental rations. Variations in the other 25% of each ration involved only zein, Solka Floc, fat, starch, and lysine.

Table 1. Ingredients common to all rations

Ingredients	lb.
Ground corn	10.0
Ground wheat	20.965
Wheat bran	4.0
Wheat shorts	11.25
Dehydrated alfalfa meal	2.0
Soybean meal (44% protein)	18.0
Meat meal (55% protein)	4.0
Herring meal (72% protein)	1.0
Ground limestone	1.5
Iodized salt	0.5
Manganese sulfate	0.025
Chromic oxide bread*	1.0
Zinc oxide	0.01
Vitamin mix**	0.75

*Chromic oxide bread (Kane et al., 1950). Supplied 0.3% chromic oxide to rations.

**Vitamins and antibiotics were added at the following levels per pound of ration: vitamin A, 1135 IU; vitamin D_3, 375 ICU; vitamin E, 5 IU; vitamin B_{12}, 0.003 mg; 25% choline chloride, 341 mg; riboflavin, 1.5 mg; calcium pantothenate, 5 mg; niacin, 10 mg; folic acid, 0.5 mg; and penicillin, 2 mg.

The C/P ratios (metabolizable energy) for the three energy levels were calculated to be 60, 70, and 80 in Trial I; 51, 60, and 69 in Trial II; and 45, 52, and 60 in Trial III. Thus, each trial had a ration containing a C/P ratio of 60 which is considered to be close to the optimum.

The level of lysine in all basal rations was calculated to be 0.9%. At each different protein and energy level, supplemental lysine was added to provide experimental rations containing levels of 0.9, 1.0, 1.1, and 1.2% of lysine. These levels were selected on the assumption that the optimum Calorie:lysine ratio is 1200 kcal of metabolizable energy per 1% of lysine. The ratio selected was based on a requirement of 1% of lysine in a ration containing 20% of protein (National Research Council, National Academy of Sciences, 1960) with an optimum C/P ratio of 60 (Hill and Renner, 1957). When this was applied to the levels of energy used in the trials it was calculated that 0.9, 1.05, and 1.2% of lysine would be optimum for rations containing energy levels of 1080, 1260, and 1440 kcal of metabolizable energy per lb., respectively.

Analytical procedures

Metabolizable energy, protein, and lysine levels were determined on each experimental ration.

Protein determination - The nitrogen content was determined by the Kjeldahl method (AOAC, 1960). Protein level was calculated using a conversion factor of 6.25.

Metabolizable energy determination - The method for determining metabolizable energy was the same as that used by Hill and Anderson (1958) with slight modifications. Details of the procedure are included in Appendix A.

Lysine determination - Lysine content of the samples was determined microbiologically following acid hydrolysis. The procedures used were the same as those outlined by Clandinin (1949) using Leuconostoc mesenteroides P60 as the test organism.

Statistical analysis

The data on average body weight and efficiency of feed conversion were analyzed statistically using analysis of variance and Duncan's new multiple range test (Steel and Torrie, 1960). A probability of 0.05 was selected as the point of significance.

Trial I

Object

To study the effects of varying the level of energy in an 18% protein starter on the lysine requirement of the chick.

Experimental

The rations used in this trial were calculated to contain 18% protein. As indicated under "Experimental (General)", three metabolizable energy levels were employed; 1080, 1260, and 1440 kcal/lb. and at each energy level four levels of lysine feeding were studied; 0.9, 1.0, 1.1, and 1.2%. Details on the rations used are found in Table 2.

Results and Discussion

As will be noted in Table 2, determined metabolizable energy values were slightly higher than anticipated. The result could perhaps be accounted for on the basis of error inherent in the use of literature values for ration composition calculations and on the basis of the effect of ingredient interaction on metabolizable energy values. Changing the level of lysine at any one energy level did not appear to have any consistent effect on the metabolizable energy value of the ration. All rations were about 1.5% higher in protein content than expected. Determined lysine values checked surprisingly well with calculated figures, particularly when one considers that the calculated figures were based on literature values and that the microbiological assay procedure is accurate only to $\pm 5\%$.

Table 2. Composition of rations used in Trial I

	Treatments											
	Group A				Group B				Group C			
Ingredients	1	2	3	4	1	2	3	4	1	2	3	4
Common ingredients*	75	75	75	75	75	75	75	75	75	75	75	75
Cornstarch	9	9	9	9	10.25	10.25	10.25	10.25	11.75	11.75	11.75	11.75
Solka Floc	12	12	12	12	6	6	6	6				
Fat (feed-grade)	2	2	2	2	6.75	6.75	6.75	6.75	11.25	11.25	11.25	11.25
Lyamine (20% lysine)		0.5	1	1.5		0.5	1	1.5		0.5	1	1.5
Shorts	2	1.5	1	0.5	2	1.5	1	0.5	2	1.5	1	0.5
Analyses (calculated)												
Metabolizable energy (kcal/lb.)	1080				1260				1440			
Protein (%)	18				18				18			
Lysine (%)	0.9	1.0	1.1	1.2	0.9	1.0	1.1	1.2	0.9	1.0	1.1	1.2
Analyses (determined)												
Metabolizable energy (kcal/lb.)	1196	1192	1170	1165	1396	1389	1385	1412	1573	1580	1592	1572
Protein (%)	19.7	19.8	19.8	19.8	19.8	19.8	19.8	19.7	19.4	19.4	19.4	19.4
Lysine (%)	0.83	0.94	1.09	1.17	0.85	0.96	1.07	1.18	0.89	0.98	1.09	1.21

*See Table 1.

In this trial (Table 3), increasing the C/P ratio from 60 to 70 to 80 or increasing the lysine content of the rations at the three energy levels from 0.9 to 1.2% did not improve growth rate ($P<0.05$). As a matter of fact, at the higher energy levels, there was some indication that the highest level of lysine decreased growth rate, although as previously indicated the depression was not statistically significant.

Energy level of the ration was found to have a highly significant effect on feed efficiency ($P<0.01$). Improvement in feed efficiency was greatest in the treatments receiving the highest energy level. Percentage of lysine in the diet had no significant effect on feed conversion.

Summary

1) Increasing the metabolizable energy content of an 18% protein starter from 1080 to 1260 to 1440 kcal/lb. or increasing the lysine content of rations at these three energy levels from 0.9 to 1.2% had no significant effect on growth rate of chicks to four weeks of age.

2) Level of energy in the ration was found to affect feed conversion in a highly significant manner. Percentage of lysine in the diet, however, did not affect feed conversion significantly.

3) Changes in the lysine content of the ration did not appear to affect the metabolizable energy value of the ration.

Table 3. Effect of treatments in Trial I

	Treatments											
	Group A				Group B				Group C			
	1	2	3	4	1	2	3	4	1	2	3	4
Calorie:protein ratio (calculated)	60				70				80			
Calorie:protein ratio (determined)	60.7	60.2	59.1	58.8	70.5	70.2	70.0	71.7	81.1	81.4	82.1	81.0
% Lysine/therm ME/lb. feed	0.70	0.80	0.92	0.99	0.61	0.69	0.77	0.85	0.56	0.62	0.69	0.77
Average body weight at 4 weeks (g)	368	372	383	369	376	401	388	358	400	413	396	367
Grams feed/gram gain	2.23	2.15	2.17	2.19	2.13	2.00	1.99	1.94	1.88	1.88	1.88	1.91

Trial II

Object

To study the effects of varying the level of energy in a 21% protein starter on the lysine requirement of the chick.

Experimental

The rations used in this trial differed from those used in Trial I in that they contained 21% rather than 18% protein. The additional protein was supplied by zein in order to cause a minimum change in the lysine content of the rations. The levels of metabolizable energy and lysine used in Trial II were similar to those used in the previous trial. Details on the rations are presented in Table 4.

Results and Discussion

As was the case in Trial I, determined metabolizable and protein values were slightly higher than expected by calculation from literature values (Table 4). Percentage lysine in each of the rations checked quite closely with calculated values. Level of lysine in the rations, again, had no effect on the metabolizable energy content.

Data collected on the effect of treatments on growth rate and feed conversion are summarized in Table 5. It will be noted that growth and feed conversion obtained in this trial were inferior to that obtained in Trial I, in spite of the fact that the protein content of the rations used in this trial averaged 3% higher than in the rations used in Trial I. It was also observed that increasing the C/P ratio from 51 to 60 to 69 or increasing the lysine content from 0.9 to 1.2% did not improve growth rate to four weeks of age, significantly.

Table 4. Composition of rations used in Trial II

	Treatments											
	Group A				Group B				Group C			
Ingredients	1	2	3	4	1	2	3	4	1	2	3	4
Common ingredients*	75	75	75	75	75	75	75	75	75	75	75	75
Cornstarch	6.25	6.25	6.25	6.25	7.75	7.75	7.75	7.75	10	10	10	10
Zein	3	3	3	3	3	3	3	3	3	3	3	3
Solka Floc	12	12	12	12	6	6	6	6				
Fat (feed-grade)	1.75	1.75	1.75	1.75	6.25	6.25	6.25	6.25	11	11	11	11
Lyamine (20% lysine)		0.5	1	1.5		0.5	1	1.5		0.5	1	1.5
Shorts	2	1.5	1	0.5	2	1.5	1	0.5	2	1.5	1	0.5
Analyses (calculated)												
Metabolizable energy (kcal/lb.)	1080				1260				1440			
Protein (%)	21				21				21			
Lysine (%)	0.9	1.0	1.1	1.2	0.9	1.0	1.1	1.2	0.9	1.0	1.1	1.2
Analyses (determined)												
Metabolizable energy (kcal/lb.)	1080	1155	1167	1129	1336	1382	1404	1332	1497	1498	1499	1501
Protein (%)	21.9	22.1	21.8	21.6	22.3	21.7	21.6	21.7	21.6	21.5	21.5	21.7
Lysine (%)	0.94	1.00	1.11	1.20	0.95	1.04	1.12	1.22	0.91	1.01	1.09	1.20

*See Table 1.

Table 5. Effect of treatments in Trial II

	Treatments											
	Group A				Group B				Group C			
	1	2	3	4	1	2	3	4	1	2	3	4
Calorie:protein ratio (calculated)	51				60				69			
Calorie:protein ratio (determined)	49.4	52.2	53.5	52.3	59.9	63.6	64.9	61.5	69.3	69.6	69.7	69.2
% Lysine/therm ME/lb. feed	0.83	0.88	0.98	1.06	0.70	0.76	0.82	0.89	0.61	0.67	0.73	0.80
Average body weight at 4 weeks (g)	333	308	328	329	341	340	354	332	338	322	324	339
Grams feed/gram gain	2.25	2.33	2.20	2.26	2.05	1.99	1.93	2.03	1.90	1.88	1.87	1.84

Feed conversion, as in Trial I, was highly significantly ($P<0.01$) affected by the energy level of the rations. The best feed efficiency was obtained from the rations containing the highest level of energy, Group C. Varying the level of lysine in the diets, however, did not affect feed conversion significantly.

Summary

1) Increasing the metabolizable energy content of a 21% protein starter from 1080 to 1260 to 1440 kcal/lb. or increasing the lysine content of the rations at these three energy levels from 0.9 to 1.2% had no significant effect on growth rate of chicks to four weeks of age.

2) Energy level of the diet was found to have a highly significant effect on feed conversion. Percentage of lysine in the diet, however, did not affect feed conversion significantly.

3) Metabolizable energy values were not affected by the level of lysine in the rations.

Trial III

Object

To study the effect of varying the energy level in a 24% protein starter on the lysine requirement of the chick.

Experimental

The rations used in this trial differed from those used in Trials I and II in that they contained 24% rather than 18 and 21% protein. The additional protein was, as in Trial II, supplied by zein in order to cause a minimum change in the lysine content of the rations. The levels of metabolizable energy and lysine used in Trial III were similar to those used in the previous trials. Details on the rations will be found in Table 6.

Results and Discussion

Examination of Table 6 reveals higher determined metabolizable energy values than those obtained by calculation from literature values. Percentage of protein and lysine in each of the rations checked closely with calculated values. As in the previous trials, varying the percentage of lysine in the starter did not affect the determined metabolizable energy values of the diets.

The effects of ration treatment on body weight and efficiency of feed conversion are summarized in Table 7. Increasing the energy level of the basal rations did not improve rate of growth. The addition of lysine to the basal rations containing 1260 and 1440 kcal of metabolizable energy per lb. resulted in a significant increase in rate of growth. In both cases addition of 0.1% of lysine was as effective as higher levels.

Table 6. Composition of rations used in Trial III

Ingredients	Treatments											
	Group A				Group B				Group C			
	1	2	3	4	1	2	3	4	1	2	3	4
Common ingredients*	75	75	75	75	75	75	75	75	75	75	75	75
Cornstarch	3.75	3.75	3.75	3.75	5	5	5	5	6.5	6.5	6.5	6.5
Zein	6	6	6	6	6	6	6	6	6	6	6	6
Solka Floc	12	12	12	12	6	6	6	6				
Fat (feed-grade)	1.25	1.25	1.25	1.25	6	6	6	6	10.5	10.5	10.5	10.5
Lyamine (20% lysine)		0.5	1	1.5		0.5	1	1.5		0.5	1	1.5
Shorts	2	1.5	1	0.5	2	1.5	1	0.5	2	1.5	1	0.5
Analyses (calculated)												
Metabolizable energy (kcal/lb.)	1080				1260				1440			
Protein (%)	24				24				24			
Lysine (%)	0.9	1.0	1.1	1.2	0.9	1.0	1.1	1.2	0.9	1.0	1.1	1.2
Analyses (determined)												
Metabolizable energy (kcal/lb.)	1127	1128	1116	1114	1345	1356	1351	1351	1532	1541	1529	1530
Protein (%)	24.4	24.5	24.6	24.4	24.8	24.9	25.3	24.9	24.7	24.7	26.3	24.8
Lysine (%)	0.91	1.04	1.13	1.20	0.91	1.00	1.16	1.20	0.94	1.01	1.10	1.22

*See Table 1.

Table 7. Effect of treatments in Trial III

	Treatments											
	Group A				Group B				Group C			
	1	2	3	4	1	2	3	4	1	2	3	4
Calorie:protein ratio (calculated)	45				52				60			
Calorie:protein ratio (determined)	46.2	46.1	45.3	45.6	54.3	54.5	53.4	54.3	62.1	62.4	58.2	61.8
% Lysine/therm ME/lb. feed	0.81	0.93	1.01	1.07	0.67	0.74	0.82	0.89	0.61	0.66	0.72	0.80
Average body weight at 4 weeks (g)*	326^{de}	352^{bcd}	332^{de}	343^{cd}	341^{cd}	376^{abc}	401^{a}	385^{ab}	301^{e}	382^{ab}	380^{ab}	391^{a}
Grams feed/gram gain	2.28	2.27	2.34	2.34	1.99	2.01	1.99	2.02	2.04	1.88	1.90	1.91

*Means with common letters are not significantly different from each other ($P<0.05$).

A highly significant interaction between lysine content and energy level of the ration on growth rate was noted.

As was found in Trials I and II, efficiency of feed conversion was affected by the energy content of the ration; the best feed conversion was noted at the highest energy level. Varying the lysine content of the ration did not affect feed conversion.

Summary

1) Increasing the metabolizable energy content of a 24% protein starter from 1080 to 1260 to 1440 kcal/lb. had no significant effect on growth rate of chicks to four weeks of age. Increasing the lysine content of the diet at the two higher energy levels significantly increased rate of growth; 1% of lysine was as effective as higher levels. A highly significant interaction between lysine content and energy level of the diet on growth rate was recorded.

2) Energy level of the ration had a significant effect on feed conversion. The lysine content of the diet, on the other hand, did not affect feed efficiency significantly.

3) Metabolizable energy values were unaffected by changes in the lysine content of the rations.

Trial IV

Object

In the preceding trials, the results obtained did not indicate that the lysine requirement was directly related to the metabolizable energy content of the rations. Since this was at variance with the original hypothesis, it seemed possible that the expression of this relationship might have been masked by growth being limited by a slight deficiency of some other amino acid. A review of amino acid levels in the rations used previously suggested that, of the essential amino acids except lysine, methionine and arginine were the ones most likely to be limiting. In order to determine whether or not this was, in fact, the case, the growth promotion and feed conversion effects of rations similar to those used in Trials I and III, but which were supplemented with methionine and arginine, were studied.

Experimental

Two series of rations were used in this trial. In one series the protein level (18%) was similar to that used in Trial I, while in the other the protein level (24%) was similar to that used in Trial III. As in the previous trials, the additional protein in the high-protein series of rations was supplied by zein in order to cause a minimum change in the lysine content of the rations. In each series, three energy levels (1080, 1260, and 1440 kcal/lb.) were used, and at each energy level four levels of lysine feeding (0.9, 1.0, 1.1, and 1.2%) were studied. Details on the rations used are found in Tables 8 and 9.

Table 8. Composition of rations used in Trial IV (low-protein series)

Ingredients	Treatments											
	Group A				Group B				Group C			
	1	2	3	4	1	2	3	4	1	2	3	4
Common ingredients*	75	75	75	75	75	75	75	75	75	75	75	75
Cornstarch	9	9	9	9	10.25	10.25	10.25	10.25	11.75	11.75	11.75	11.75
Solka Floc	12	12	12	12	6	6	6	6				
Fat (feed-grade)	2	2	2	2	6.75	6.75	6.75	6.75	11.25	11.25	11.25	11.25
L-lysine		0.1	0.2	0.3		0.1	0.2	0.3		0.1	0.2	0.3
DL-methionine	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
L-arginine	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Shorts	1.65	1.55	1.45	1.35	1.65	1.55	1.45	1.35	1.65	1.55	1.45	1.35
Analyses (calculated)												
Metabolizable energy (kcal/lb.)	1085				1265				1445			
Protein (%)	18				18				18			
Lysine (%)	0.9	1.0	1.1	1.2	0.9	1.0	1.1	1.2	0.9	1.0	1.1	1.2
Analyses (determined)												
Metabolizable energy (kcal/lb.)	1194	1180	1189	1191	1339	1328	1330	1348	1544	1546	1543	1545
Protein (%)	19.9	19.9	20.1	21.0	20.6	20.4	20.3	20.3	21.3	21.2	21.0	21.0
Lysine (%)	0.89	0.93	1.03	1.10	0.85	0.91	1.01	1.10	0.81	0.88	1.00	1.10

*See Table 1.

Table 9. Composition of rations used in Trial IV (high-protein series)

	Treatments											
	Group A				Group B				Group C			
Ingredients	1	2	3	4	1	2	3	4	1	2	3	4
Common ingredients*	75	75	75	75	75	75	75	75	75	75	75	75
Cornstarch	3.75	3.75	3.75	3.75	5	5	5	5	6.5	6.5	6.5	6.5
Zein	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5
Solka Floc	12	12	12	12	6	6	6	6				
Fat (feed-grade)	1.25	1.25	1.25	1.25	6	6	6	6	10.5	10.5	10.5	10.5
L-lysine		0.1	0.2	0.3		0.1	0.2	0.3		0.1	0.2	0.3
DL-methionine	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
L-arginine	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Shorts	0.9	0.8	0.7	0.6	0.9	0.8	0.7	0.6	0.9	0.8	0.7	0.6
Analyses (calculated)												
Metabolizable energy (kcal/lb.)	1089				1269				1449			
Protein (%)	24				24				24			
Lysine (%)	0.9	1.0	1.1	1.2	0.9	1.0	1.1	1.2	0.9	1.0	1.1	1.2
Analyses (determined)												
Metabolizable energy (kcal/lb.)	1198	1198	1205	1199	1320	1343	1339	1332	1542	1550	1544	1558
Protein (%)	27.6	26.5	26.3	27.3	26.5	26.3	27.2	27.8	27.5	27.8	27.3	27.1
Lysine (%)	0.80	0.90	1.06	1.10	0.78	0.87	1.03	1.10	0.79	1.86	1.07	1.13

*See Table 1.

Results and Discussion

As noted in the previous trials, determined metabolizable energy values (Tables 8 and 9) were higher than expected by calculation from literature values. Percentage of lysine in the rations checked quite well with calculated values, however, nitrogen analyses indicated that all rations were approximately 2% higher in protein content than anticipated by calculation. As in the previous trials, no interaction between the lysine level of the rations and metabolizable energy values for the diets was recorded.

In both the low and high-protein series, energy level was found to have a highly significant effect ($P<0.01$) on body weight at four weeks of age (Table 10). This observation is contrary to that noted in Trials I and III in which energy level was found to exert no measurable effect on growth to four weeks of age. Perhaps the supplementation of the diets in Trial IV with methionine and arginine was responsible for the differences in response to energy noted. In this connection, the possibility exists that growth in Trials I and III was limited by a partial methionine and/or arginine deficiency and, as a result, growth response from increasing the energy level of the diets was thereby prevented. Level of lysine, on the other hand, was found to have a significant effect on growth rate only in the groups that received the rations containing the highest energy levels. In this connection, 1% of lysine was found as effective for growth promotion as higher levels. This suggests that the optimum ratio between kcal/lb. of metabolizable energy and lysine is approximately 1440:1 rather than 1200:1 as was assumed when these experiments were initiated.

No increase in body weight at four weeks was observed as a

Table 10. Effect of treatments in Trial IV

	Treatments											
	Group A				Group B				Group C			
	1	2	3	4	1	2	3	4	1	2	3	4
<u>Low-protein series</u>												
Calorie:protein ratio (calculated)	60.3				70.3				80.3			
Calorie:protein ratio (determined)	60	59.3	59.2	56.7	65	65.1	65.5	66.4	72.5	72.9	73.5	73.6
% Lysine/therm ME/lb. feed	0.75	0.78	0.87	0.92	0.64	0.69	0.76	0.82	0.52	0.57	0.65	0.71
Average body weight at 4 weeks (g)*	375[a]	394[abc]	381[ab]	410[abcd]	435[cd]	454[de]	437[d]	437[d]	421[bcd]	443[d]	445[d]	490[e]
Grams feed/gram gain	2.08	2.13	2.07	2.16	1.77	1.82	1.75	1.89	1.73	1.70	1.70	1.76
<u>High-protein series</u>												
Calorie:protein ratio (calculated)	45.4				52.9				60.4			
Calorie:protein ratio (determined)	43.4	41.4	45.8	43.9	49.8	51.1	49.2	47.9	56.1	55.8	56.5	57.5
% Lysine/therm ME/lb. feed	0.67	0.75	0.88	0.92	0.58	0.65	0.77	0.82	0.51	0.56	0.69	0.73
Average body weight at 4 weeks (g)*	358[a]	370[ab]	380[abc]	370[ab]	415[cde]	409[cd]	433[de]	418[cde]	385[abc]	437[de]	440[de]	459[e]
Grams feed/gram gain	2.12	2.11	2.08	2.11	1.84	1.73	1.77	1.78	1.72	1.66	1.60	1.60

*Means with common letters are not significantly different from each other ($P<0.05$).

result of increasing the protein content of the rations from 18 to 24%. It would appear, therefore, that sufficient protein was supplied in the low-protein series to meet the chick's requirements for essential amino acids at the energy levels used in the study.

Energy and protein levels of the ration were found to have significant effects on feed conversion. Varying the lysine content of the diet, however, did not have a consistent effect on feed conversion.

Summary

1) Increasing the metabolizable energy content of 18 and 24% protein starters supplemented with methionine and arginine from 1080 to 1260 to 1440 kcal/lb. was found to have a highly significant effect ($P < 0.01$) on body weight of chicks at four weeks of age. Varying the lysine content of the diets at the three energy levels, however, only affected growth rate significantly at the highest energy levels. In this connection, 1% of lysine was found to be as effective for growth promotion as higher levels. No optimum level of lysine per therm of metabolizable energy per pound of feed was indicated by the data.

2) Energy and protein levels of the ration significantly affected feed conversion. Lysine level, however, did not appear to influence feed conversion in a consistent manner.

3) Metabolizable energy values, as in previous trials, were unaffected by the lysine content of the rations.

GENERAL DISCUSSION

In the trials reported herein, the results obtained did not generally support the hypothesis that amino acid requirements are directly related to the energy level of the ration. This is contrary to the observations of others (Rosenberg and Baldini, 1956; Schwartz et al., 1958) and does not conform with the generalization of Gordon et al. (1958) that within any one set or combination of protein supplements the proportion of energy content to protein content appears to fix amino acid requirements.

The level of lysine in the basal rations (0.9%) was such that it presumably would be adequate when the ration contained 1080 kcal of metabolizable energy per pound, provided that the original assumption that the optimum metabolizable energy:lysine ratio is 1200:1 was valid. It should then follow that when the energy content of the ration was increased, a reduction in rate of growth would occur because of decreased feed intake and reduced lysine consumption. The higher levels of energy used did result in lower feed intake and increased efficiency of feed conversion but no effect on growth rate was noted. The results would seem to imply that the optimum metabolizable energy:lysine ratio might be much higher than was assumed. This seems unlikely since other investigators (Combs, 1962; Combs and Nicholson, 1963) have suggested that the optimum metabolizable energy:lysine ratio is approximately 1250:1.

The determined levels of metabolizable energy and protein in the rations used were consistently higher than the calculated values. This suggests the need for re-evaluation of metabolizable energy values of feedstuffs being used in ration formulation in Western Canada. The

[illegible]

[illegible]

[illegible]

differences in determined protein levels from calculated values reflects the type of variability often encountered when average values for the protein content of feedstuffs are used in connection with ration formulation.

GENERAL SUMMARY

Experiments were designed to permit study of the effect of level of metabolizable energy and ratio between metabolizable energy and crude protein in chick rations on growth rate, feed conversion, and on the requirement of the chick for lysine. The results obtained indicate:

1) Changing the metabolizable energy content from 1080 to 1260 to 1440 kcal/lb. in chick starters containing 18, 21, and 24% protein in which the percentage of lysine was maintained constant (Trials I, II, and III) had no significant effect on the growth rate of chicks to four weeks of age. However, when the 18 and 24% protein starters were supplemented with methionine and arginine (Trial IV), the changes in metabolizable energy content through the range indicated above were found to have highly significant ($P<0.01$) effects on growth rate. It would therefore appear that the basic starters employed in the first three trials were slightly limiting in methionine and arginine and, as a result, prevented growth response from increases in energy content of the rations.

2) Increasing the lysine content of diets containing 18, 21, and 24% protein (Trials I, II, and III) at the three energy levels referred to above from 0.9, 1.0, 1.1, to 1.2% did not affect growth rate significantly except at the two higher energy levels in Trial III. However, when the 18 and 24% protein starters were supplemented with methionine and arginine (Trial IV), varying the lysine affected growth rate only at the highest energy level. A level of 1% lysine was found as effective for growth promotion as higher levels.

3) Increasing the energy level of the diets was found to have

significant effects on feed conversion. In general, at any one protein level, best feed conversion was obtained from the rations containing the highest energy level. Increasing the lysine content of the rations at any one protein or energy level through the range indicated above did not affect feed conversion significantly.

4) Metabolizable energy values were unaffected by variations in the lysine content of the rations.

BIBLIOGRAPHY

Almquist, H. J., and E. Mecchi. 1942. Lysine requirement of the chick. Proc. Soc. Exp. Biol. Med. 49:174.

Almquist, H. J. 1947. Evaluation of amino acid requirements by observations on the chick. J. Nutrition 34:543-564.

Almquist, H. J. 1952. Amino acid requirements of chickens and turkeys - A review. Poultry Sci. 31:966-981.

Association of Official Agricultural Chemists. 1960. Official methods of analysis. 9th ed. Washington, D.C.

Baldini, J. T., and H. R. Rosenberg. 1955. The effect of productive energy level of the diet on the methionine requirement of the chick. Poultry Sci. 34:1301-1307.

Baldini, J. T. 1961. The effect of dietary deficiency on the energy metabolism of the chick. Poultry Sci. 40:1177-1183.

Beilharz, R. G., and M. W. McDonald. 1959. The use of high quality fat and the effect of protein level in broiler diets. Poultry Sci. 38: 519-526.

Biely, J., and B. March. 1954. Fat studies in poultry. 2. Fat supplements in chick and poult rations. Poultry Sci. 33:1220-1227.

Blaylock, L. G., and L. R. Richardson. 1950. Peanut meal, mixtures of soybean and cottonseed and mixtures of soybean and peanut meals as sources of protein for baby chicks. Poultry Sci. 29:656-660.

Block, R. J., and K. W. Weiss. 1956. Amino acid handbook. Methods and results of protein analysis. Charles C. Thomas, Publisher. Springfield, Illinois.

Clandinin, D. R. 1949. The effects of methods of processing on the nutritive value of herring meals. Poultry Sci. 28:128-133.

Combs, G. F., and G. L. Romoser. 1955. A new approach to poultry feed formulation. Feed Age 5, No. 3, 50-58.

Combs, G. F. 1962. The interrelationship of dietary energy and protein in poultry nutrition, p. 127-147. In J. T. Morgan and D. Lewis (ed.) Nutrition of pigs and poultry. Butterworths, London.

Combs, G. F., and J. L. Nicholson. 1963. Sulfur amino acid and lysine requirements of broiler chickens. Poultry Sci. 42:1261.

Donaldson, W. E., G. F. Combs, and G. L. Romoser. 1956. Studies on energy levels in poultry rations. I. The effect of calorie-protein ratio of the ration on growth, nutrient utilization and body composition of chicks. Poultry Sci. 35:1100-1105.

Edwards, H. M., Jr., L. C. Norris, and G. F. Heuser. 1956. Studies on the lysine requirement of chicks. Poultry Sci. 35:385-390.

Fraps, G. S., and E. C. Carlyle. 1939. The utilization of the energy of feed by growing chickens. Texas Agr. Exp. Sta. Bull. 571.

Fraps, G. S., E. C. Carlyle, and J. F. Fudge. 1940. Metabolizable energy of some chicken feeds. Texas Agr. Exp. Sta. Bull. 589.

Fraps, G. S., and E. C. Carlyle. 1942. Productive energy of some feeds as measured by gains of energy of growing chickens. Texas Agr. Exp. Sta. Bull. 625.

Fraps, G. S. 1945. Utilization of metabolizable energy by growing chicks. Arch. Biochem. 8:21-27.

Fraps, G. S. 1946. Composition and productive energy of poultry feeds and rations. Texas Agr. Exp. Sta. Bull. 678.

Gordon, R. S., K. H. Maddy, and L. J. Machlin. 1958. Proportion of dietary energy content to protein content as the prime determinant of the amino acid requirements of the young chicken. Poultry Sci. 37:1208.

Grau, C. R. 1948. Effect of protein level on the lysine requirement of the chick. J. Nutrition 36:99-108.

Grau, C. R., and M. Kamei. 1950. Amino acid imbalance and the growth requirements for lysine and methionine. J. Nutrition 41:89-101.

Griminger, P., H. M. Scott, and R. M. Forbes. 1956. The effect of protein level on the tryptophan requirement of the growing chick. J. Nutrition 59:67-76.

Griminger, P., and H. M. Scott. 1959. Growth rate and lysine requirement of the chick. J. Nutrition 68:429-442.

Guttridge, D. 1957. Broiler nutrition with reference to energy:protein ratios and their influence on growth, food conversion and profit. World's Poultry Sci. J. 13:280-283.

Hill, F. W., and L. M. Dansky. 1950. Studies of the protein requirements of chicks and its relation to dietary energy level. Poultry Sci. 29:763.

Hill, F. W., and L. M. Dansky. 1954. Studies of the energy requirements of chickens. 1. The effect of dietary energy level on growth and feed consumption. Poultry Sci. 33:112-119.

Hill, F. W., and R. Renner. 1957. Metabolizable energy values of feedstuffs for poultry and their use in formulation of rations. Proc. Cornell Nutrition Conference for Feed Manufacturers.

Hill, F. W., and D. L. Anderson. 1958. Comparison of metabolizable energy and productive energy determination with growing chickens. J. Nutrition 64:587-603.

Kane, E. A., W. C. Jacobson, and L. A. Moore. 1950. A comparison of techniques used in digestibility studies with dairy cattle. J. Nutrition 41:583-596.

Leong, K. C., M. L. Sunde, H. R. Bird, and C. A. Elvehjem. 1955. Effect of energy:protein ratio on growth rate, efficiency, feathering and fat deposition in chickens. Poultry Sci. 34: 1206-1207.

Milligan, J. L., L. J. Machlin, H. R. Bird, and B. W. Heywang. 1951. Lysine and methionine requirement of chicks fed practical diets. Poultry Sci. 30:578-586.

National Research Council, National Academy of Sciences (US). 1960. Nutrient requirements for domestic animals. No. 1. Nutrient Requirements of Poultry.

O'Neil, J. B., J. Biely, G. C. Hodgson, J. R. Aitken, and A. R. Robblee. 1962. Protein energy relationships in the diet of the chick. Poultry Sci. 41:739-745.

Panda, J. N., and G. F. Combs. 1950. Studies on the energy requirement of the chick for rapid growth. Poultry Sci. 29:774-775.

Peterson, D. W., G. R. Grau, and N. F. Peek. 1954. Growth and food consumption in relation to dietary levels of protein and fibrous bulk. J. Nutrition 52:241-257.

Richardson, C. E., A. B. Watts, and E. A. Epps. 1956. Energy studies with broilers: the effect of using various fibrous feedstuffs, with and without added fat, in a practical broiler ration. Poultry Sci. 35:1167-1168.

Richardson, L. R., and L. G. Blaylock. 1950. Vitamin B_{12} and amino acids as supplements to soybean oil meal and cottonseed meal for growing chicks. J. Nutrition 40:169-176.

Rosenberg, H. R., and J. T. Baldini. 1956. Effect of dietary protein level on the methionine-energy relationship in broiler diets. Poultry Sci. 35:1168.

Schwartz, H. G., M. W. Taylor, and H. Fisher. 1958. The effect of dietary energy concentration and age on the lysine requirement of growing chicks. J. Nutrition 65:25-37.

Scott, H. M., L. D. Matterson, and E. P. Singsen. 1947. Nutritional factors influencing growth and efficiency of feed utilization. 1. The effect of the source of carbohydrate. Poultry Sci. 26:554.

Sibbald, I. R., S. J. Slinger, and G. C. Ashton. 1961a. Factors affecting the metabolizable energy content of poultry feeds. 2. Variability in the M.E. values attributed to samples of tallow and undegummed soybean oil. Poultry Sci. 40:303-308.

Sibbald, I. R., S. J. Slinger, and G. C. Ashton. 1961b. The influence of dietary calorie:protein ratios on the weight gain and feed efficiency of growing chicks. Poultry Sci. 40:308-313.

Steel, R. G. D., and J. H. Torrie. 1960. Principles and procedures of statistics. McGraw-Hill Book Company, Inc., New York.

Sunde, M. L. 1956. A relationship between protein level and energy level in chick rations. Poultry Sci. 35:350-354.

Vondell, R. M., and R. C. Ringrose. 1958. The effect of protein and fat levels and calorie to protein ratio upon performance of broilers. Poultry Sci. 37:147-151.

Williams, M. A., and C. R. Grau. 1953. Effects of variations in dietary energy on food intake and utilization of lysine deficient protein. Poultry Sci. 32:928.

APPENDIX A

Metabolizable Energy Determination

The method followed was essentially that reported by Hill and Anderson (1958).

Procedure

Chromic oxide was used as an index substance. It was added to all experimental diets at 0.3%.

Samples of excreta from each experimental treatment were collected on each of the last three days of the experimental period. They were frozen in plastic bags and stored at 0 F. The three-day excreta collections were pooled and, following the addition of some water, were homogenized. The homogenized samples were next brought to pH 5.7 by the addition of approximately 2.2 ml 6N H_2SO_4 per 100 g of frozen excreta. Aliquots of the acidified, homogenized excreta were dried at 150 F for 30 hours. They were finally made ready for analysis by grinding through a 20 mesh screen.

Feed ingredients and rations for analysis were ground through a 20 mesh screen.

Energy determinations were made with a Parr Oxygen Bomb Calorimeter while nitrogen determinations were done by the Kjeldahl method and chromic oxide by the method of Hill and Anderson (1958) (see Appendix B for details of the latter method).

Metabolizable energy was computed as follows:

Metabolizable energy per gram diet = energy per gram diet - energy in excreta per gram diet - 8.22 x nitrogen retention per gram diet, where:

Energy per gram diet = calories combustible energy per gram diet,

Energy in excreta per gram diet = calories combustible energy per gram excreta $\times \dfrac{Cr_2O_3 \text{ per gram diet}}{Cr_2O_3 \text{ per gram excreta}}$,

Factor 8.22 = combustible energy value of uric acid per gram of nitrogen, and

Nitrogen retention per gram diet = nitrogen per gram diet - nitrogen per gram excreta $\times \dfrac{Cr_2O_3 \text{ per gram diet}}{Cr_2O_3 \text{ per gram excreta}}$.

APPENDIX B

Chromic oxide determination

The method followed was that reported by Hill and Anderson (1958).

Reagents

1) Concentrated nitric acid

2) Concentrated sulfuric acid

3) Digestion mixture prepared as follows:

Dissolve 10 g sodium molybdate in 150 ml distilled water. Add 150 ml concentrated sulfuric acid slowly. After cooling in an ice bath, add 200 ml 70% perchloric acid with stirring.

Procedure

1) Weigh sample, wrap in filter paper, and place in micro-Kjeldahl flask marked at 110 ml. When 0.3% chromic oxide is used in the diet, 3 g of diet and 1.5 g of excreta are suitable samples.

2) Add 10 ml concentrated HNO_3 and allow to stand overnight.

3) Heat to dryness on a micro-Kjeldahl digestion apparatus.

4) Add 15 ml digestion mixture and heat on micro-Kjeldahl digestion apparatus until color changes from green to yellow, orange, or red. Rotation of the flasks is essential to ensure complete destruction of organic matter and complete oxidation of Cr_2O_3 to chromate.

5) Remove flasks, chill in cold water, dilute with 60 ml distilled water, add 20 ml concentrated H_2SO_4, cool, dilute to volume (120 ml), mix, and allow to stand overnight to permit insolubles to settle.

6) Determine optical density at 450 mμ using a Beckman DU Spectrophotometer. Compute chromic oxide content from a reference curve

covering the range 0 to 250 μg of chromic oxide per milliliter. (The reference curve used in this study had the form $x = 48.52\ y$, where x = milligrams chromic oxide per 110 ml, and y = optical density.)

B29840